

Relative Susceptibility of Acid-Fast and Non-Acid-Fast Bacteria to Ultraviolet Light

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Mycobacterium tuberculosis strain Erdman, *M. bovis* (BCG), and *M. phlei* showed a 1- to 2-log drop in viability after exposure to ultraviolet light compared to a 5-log drop over the same period for *Staphylococcus albus*, *Listeria monocytogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, and *Serratia marcescens*. *L. monocytogenes* showed an initial resistance to ultraviolet inactivation, but later the inactivation rate increased sharply. The significance of these findings with regard to the use of *S. marcescens* as a test organism for determining the bactericidal efficiency of ultraviolet lamps used to sterilize equipment contaminated with tubercle bacilli is discussed.

Ultraviolet light is routinely used to sterilize glove boxes and fume hoods contaminated with virulent bacteria, fungi, and viruses. However, the amount of bactericidal radiation slowly declines with time, although the total output by the lamp may not be greatly affected. Therefore, it is prudent to test the bactericidal capacity of the lamp directly from time to time under normal working conditions. Although a great deal of information now exists on the bactericidal capacity of ultraviolet light for a wide variety of organisms (1, 5), no quantitative data appear to exist regarding the rate of inactivation of mycobacteria. Ultraviolet light effectively kills airborne tubercle bacilli in hospital wards (3), and, in fact, exposure to high doses of such radiation may well be the method of choice for the sterilization of air. However, quantitative inactivation data on this point do not appear to exist (4).

For technical reasons, the effectiveness of ultraviolet lights is usually checked with suspensions or aerosols of *Escherichia coli* or *Serratia marcescens*. It is assumed that extrapolation of these data to include *Mycobacterium tuberculosis* is justifiable. In the absence of direct quantitative data, however, this practice may be questioned. For this reason, the rate of ultraviolet inactivation of three mycobacteria was compared directly with several gram-negative bacteria under standardized conditions.

MATERIALS AND METHODS

Organisms. *Staphylococcus albus* was isolated from normal human skin. *Listeria monocytogenes* was a mouse virulent strain maintained at the Trudeau

Institute by continuous mouse passage (2). *M. tuberculosis* strain Erdman [Trudeau mycobacterial culture (TMC) no. 107], *M. bovis* BCG strain Montreal (TMC no. 1008), and *M. phlei* (TMC no. 1523) were maintained on American Trudeau Society (ATS) egg slants, subcultured at 14-day intervals. *E. coli* ATCC 11775 and *Serratia marcescens* ATCC 13880 were obtained from the American Type Culture Collection, Rockville, Md. *Salmonella enteritidis* NCTC 5694 and *Pseudomonas aeruginosa* NCTC 6750 were obtained from the National Collection of Type Cultures, Collindale, United Kingdom. The *Serratia* cultures were incubated in tryptone soy broth at 25 C for 48 hr. All other cultures were incubated at 37 C for 18 hr.

Ultraviolet irradiation. Mycobacterial growth was removed from the ATS slant, homogenized in 1% buffered gelatin (pH 6.8), and standardized turbidimetrically. Microscopic examination showed this to be predominantly a single-cell suspension with very few clumps of organisms. Suitable 10-fold saline dilutions were spotted onto dried Middlebrook 7H10 agar plates. The non-acid-fast cultures were similarly inoculated onto tryptone soy agar plates. Duplicate, unirradiated control plates were placed in an incubator at 37 C, whereas similar plates were exposed, for increasing time periods, to irradiation from a Westinghouse 14-w cold cathode mercury vapor lamp. The plates were exposed to an output of 40 $\mu\text{W}/\text{cm}^2$ at a distance of 40 cm. The mycobacteria were incubated for 21 days in plastic bags in a light-tight box to prevent photoreactivation. The other organisms were incubated in the dark at appropriate temperatures for 3 days, and the colonies were counted. The percentage inactivation was calculated from the number of colonies developing on the irradiated, compared with the unirradiated, control plates. Wherever possible, comparisons were made between plates initially inoculated with 200 to 400 organisms.

RESULTS

The results of the inactivation experiments for the gram-positive organisms are shown in Fig. 1. The three strains of mycobacteria were consider-

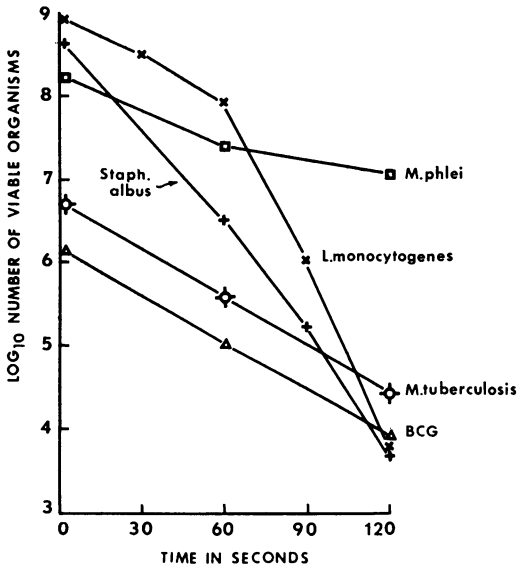


FIG. 1. Inactivation of *Staphylococcus albus* (+), *Listeria monocytogenes* (×), *Mycobacterium tuberculosis* (◇), *M. bovis BCG*, (△), and *M. phlei* (□) exposed to ultraviolet light.

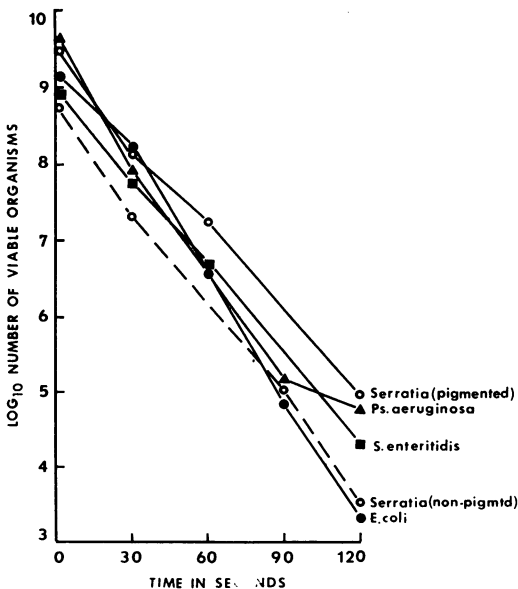


FIG. 2. Inactivation of *Escherichia coli* (●), *Pseudomonas aeruginosa* (▲), *Salmonella enteritidis* (■), *Serratia marcescens* (○), and its achromogenic mutant (○—) exposed to ultraviolet light.

ably more resistant to inactivation than any of the other bacteria tested. After 2 min of exposure, colony counts indicated that only approximately 1% of the organisms were still viable. There did not appear to be any difference in the sensitivity of the highly virulent human strain (Erdman) and the attenuated BCG strain. The saprophytic *M. phlei* appeared, if anything, to be slightly more resistant to irradiation than the other two strains. In three duplicate experiments, *L. monocytogenes* showed an early resistance to ultraviolet inactivation. However, after about the first minute of exposure, the rate of inactivation increased. The viability of *S. albus* declined at a rapid rate throughout the exposure period.

Compared with mycobacteria, all of the gram-negative bacteria tested were twice as sensitive to ultraviolet inactivation since the colony counts were reduced to approximately 1% of the controls after only 1 min of exposure to the same amount of irradiation (Fig. 2). The pigmented strain and a colorless variant of *S. marcescens* were equally sensitive to ultraviolet light.

DISCUSSION

The bactericidal quality of sunlight and ultraviolet light has been well known for nearly a century (1). Both industrially and in the laboratory, ultraviolet light is used to reduce aerial contamination and to sterilize infected surfaces (5). It has often been the practice to check equipment used in potentially hazardous operations involving aerosols of pathogenic bacteria, or for manipulations such as homogenation of tuberculous tissue, by exposing the equipment to dense suspensions of *S. marcescens* and then attempting to recover the tracer strain after irradiation. Such tests of the bactericidal efficiency of the ultraviolet lamps used in both glove boxes and fume hoods were conducted periodically at this laboratory. During one such routine check of an aerogenic infection apparatus, it was observed that a *Listeria* culture appeared to survive a period of irradiation which had previously been found to inactivate more than 99.9% of a suspension of *S. marcescens* (G. P. Kubica, unpublished data). Since the same equipment was also used for the aerogenic challenge of animals with the highly virulent Erdman strain of *M. tuberculosis*, it was thought prudent to check directly the relevance of the *Serratia* inactivation data by using both *L. monocytogenes* and *M. tuberculosis* suspensions. The present data show that a dose of radiation capable of preventing the growth of 99% of the colonies of a suitable suspension of *S. marcescens* killed less than 90% of an *M. tuberculosis* or *L. monocytogenes* population in the same time. This

means that the tubercle bacillus is two to three times as resistant to inactivation by ultraviolet light as *E. coli* or *S. marcescens*. Although this difference in sensitivity may have little real significance under most working conditions in which time of exposure is not a critical factor, it may well be important when low dosage levels are used or when exposure times are unavoidably brief. Certainly, this difference should be borne in mind when using this means of sterilizing heavily infected equipment or highly contaminated air.

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